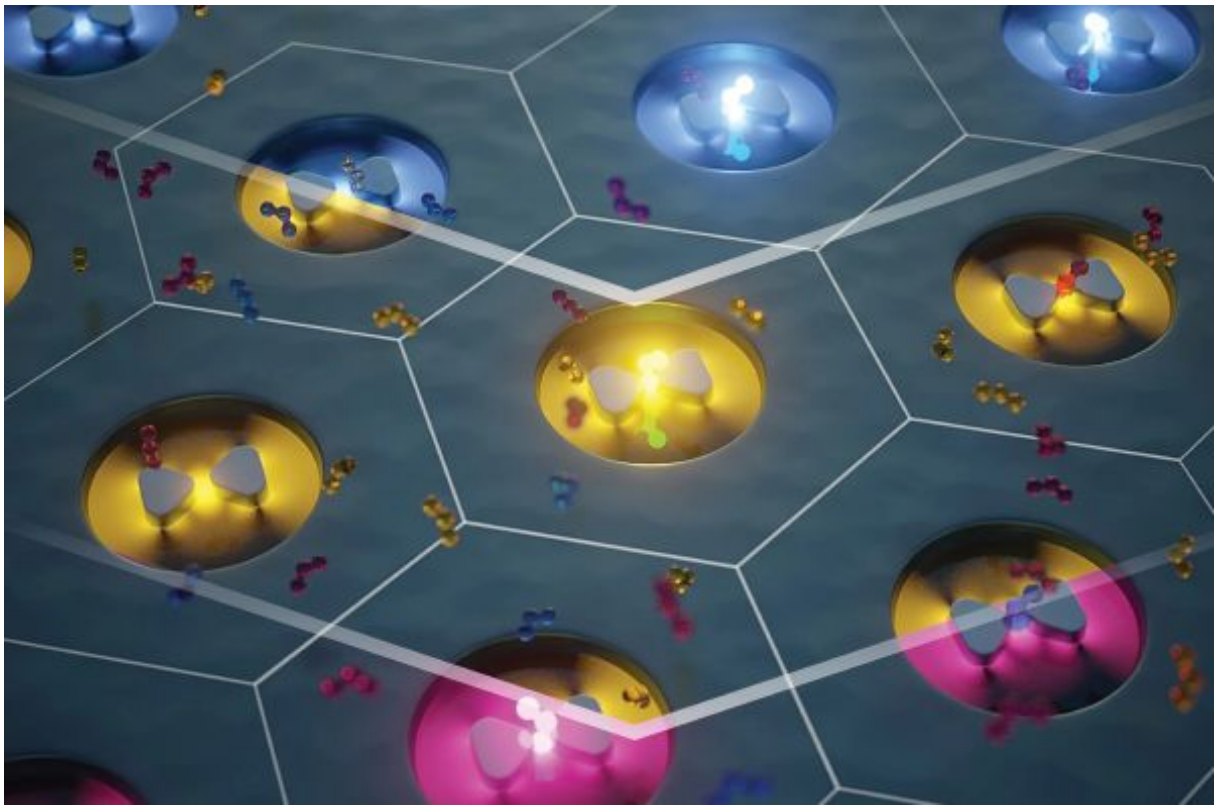

Novel nanoantennas enable sensitive multicolor single-molecule detection with unprecedented throughput

ICFO researchers have developed a nanoantenna platform capable of enhancing fluorescence emission across the entire visible spectrum, enabling multicolor single-molecule detection at micromolar concentrations. The proposed method can also address more than a thousand nanoantennas in parallel, speeding up data acquisition times.

August 26, 2024

Unravelling the intricacies of biological processes at the single-molecule level is crucial for capturing the heterogeneity of biological systems and expanding our knowledge of their biochemical dynamics. The complexity inherent in the biological environment, though, demands very sensitive biosensing techniques, which commonly capture the fluorescence light emitted by the desired biomolecules or their fluorescent labels. Maximizing their fluorescence brightness is essential to ensure their highly sensitive detection. To date, the

investigation of the interactions between different biomolecules with single-molecule sensitivity at typical concentrations found within living organisms is a major challenge that conventional methods face.

So-called plasmonic nanoantennas can provide single-molecule sensitivity under these conditions as they can significantly increase the brightness of a nearby fluorescent molecule. However, detecting single molecules with such nanoantennas under biologically relevant conditions comes with two major challenges.

On the one hand, the detection of different molecular species that emit fluorescence light at different colors (wavelenghts), requires strong fluorescence enhancement across various spectral regions. This kind of studies, known as multicolor experiments, require nanoantennas with a spectrally wide resonance, which typically results in weak amplification of the fluorescence emission and consequently hampers the detection sensitivity.

On the other hand, biosensing applications often require single-molecule sensitivity at micromolar concentrations, the concentration level at which many biological molecules, interactions, and reactions occur. Unfortunately, at such high concentrations, the single-molecule response is obscured by the strong background signal generated by the large number of surrounding molecules.

Taking both problem areas into account, the goal becomes clear: to develop a nanoantenna design that simultaneously enhances the target molecules' fluorescence and reduces the background noise across the entire visible spectrum.

Now, ICFO researchers **Ediz Kaan Herkert**, **Lukas Lau**, **Roger Pons Lanau**, led by **ICREA Prof. Maria F. Garcia-Parajo** have developed a nanoantenna platform that successfully tackles these issues. It enhances fluorescence across the entire visible spectrum and at the same time reduces background fluorescence, enabling multicolor single-molecule detection at micromolar concentration. Importantly, it additionally shows a 1000-fold increase in the number of antennas that can be addressed in parallel, which facilitates a rapid screening of samples, leading in turn to high throughput (that is, the amount of information that can be processed in a given temporal window). The results have been published in ACS Applied Materials & Interfaces.

New nanoantenna platform: High-density Hexagonal Closed-Packed Antenna-in-Box

Antenna-in-box (AiB) platforms, consisting of a nanoantenna within a nanoaperture, have been successfully applied to highly relevant biosensing tasks due to their combination of strong fluorescence background reduction and fluorescence emission enhancement. Despite their potential for biosensing applications that has been refined throughout the years, two major challenges remain: traditional AiBs are unsuitable for multicolor experiments and their throughput is slowed down by their sequential point-by-point readout (that is, only one antenna-in-box can be measured at a time).

The design developed at ICFO overcomes these challenges by introducing high-density

hexagonal closed-packed AiBs (HCP-AiBs) made of aluminum. The researchers' approach involved placing as many AiBs as close to each other as possible. In contrast to the commonly chosen square-like arrangement, a hexagonal close-packing allowed them to maximize the number of AiBs within a given space.

The high packing density significantly increased the number of AiBs that could be detected simultaneously. In the end, **the team managed to address over 1000 AiBs in parallel, consequently speeding up the data acquisition times**. i¿½For biosensing applications, th s means that **the number of analytes to be detected could be increased by three-orders of magnitude**, which is a unique accomplishment in the fieldi¿½, shares ICREA Prof. Maria . Garcia-Paraj . Moreover, choosing aluminum over gold (the material traditionally employed f r nanoantennas) was pivotal in **achieving broadband resonances covering the entire visible range**. The team then designed a custom microscope with three excitation channels, each of them successfully capturing the fluorescence emission of a different molecular species.

Multicolor fluorescence enhancement and parallelization of the readout method unlocked

The accomplishment of multicolor fluorescence enhancement and parallelization of the readout method are unique milestones that were very hard to experimentally demonstrate in the past, but the ICFO team has now succeeded in unlocking them efficiently. i¿½That is w y I consider our results a major step forward towards true real-world applications f nanoantennas in the field of biosensingi¿½, shares Ediz Herkert, first author of the artic e. But the work is not finished yet, as there is always room for improvement. i¿½The nex steps in the field should focus on developing a more time- and cost-efficient cleanroom proc ss for producing this type of hexagonal antenna arrays, and demonstrating that the mul icolor single-molecule sensitivity of HCP-AiBs can be achieved in biologically relevant cond tions, including living cellsi¿½, explains Herkert. ICREA Prof. Maria Garcia-Parajo sees great potential in their discovery as wel : i¿½Our HCP-AiBs could be used in multicolor biosensing applications to study interacti ns between different proteins on the cell membrane or to monitor biomolecular binding kin tics, both with enhanced single-molecule detection sens

Reference:

Ediz Kaan Herkert, Lukas Lau, Roger Pons Lanau, and Maria F. Garcia-Parajo. ACS Applied Materials & Interfaces **2024** 16 (31), 41271-41280
DOI: 10.1021/acsami.4c04744

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